REMARKS

Claims 1-42 have been cancelled and replaced with new Claims 43-53. New Claim 43 combines old Claims 35-37 and adds the limitation "wherein the polypeptide is administered to the chronic skin ulcer for a duration sufficient to achieve at least 80% closure of the chronic skin ulcer". Support for this limitation is found in the Exemplification, e.g., page 13, lines 10-12 and page 13, lines 23-25. New Claims 44-48 correspond to old Claims 38-42.

Interview Summary

Examiners Weber and Mondesi are thanked for granting the interview on September 21, 2005 at the USPTO and for their helpful comments and suggestions during the interview. As to the substance of the interview, Applicants refer to the Interview Summary supplied by the Examiner.

Rejection of Claims 5, 8 and 14 Under 35 U.S.C. § 112, First Paragraph

Claims 5, 8 and 14 have been rejected under 35 U.S.C. § 112, first paragraph, as they are said to fail to comply with the written description requirement. The Examiner states that Applicant has failed to provide a written description of the substitutions and deletions that would provide adequate support of the claimed invention.

Claims 1-42 have been replaced with new Claims 43-53. It is respectfully submitted that the rejection is overcome with this amendment.

Rejection of Claims 1-6, 8-9, 14-15, 26, 28, 35, 37 and 40-41 Under 35 U.S.C. § 112, Second Paragraph

Claims 1-6, 8-9, 14-15, 26, 28, 35, 37 and 40-41 have been rejected under 35 U.S.C. § 112, second paragraph, as they are said to be indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. The Examiner states that Applicant has not stated the nature or the end result of the response initiated by the agonist.

New Claim 43 recites that the "polypeptide is administered to the chronic skin ulcer for a duration sufficient to achieve at least 80% closure of the chronic skin ulcer". With this amendment, it is believed that the rejection has been overcome.

Rejection of Claims 1-6, 8-9, 14, 15, 26, 28, 35, 37 and 40-41 Under 35 U.S.C. § 102(b)

Claims 1-6, 8-9, 14, 15, 26, 28, 35, 37 and 40-41 have been rejected under 35 U.S.C. § 102(b), as they are said to be anticipated by Carney et al. (US 5,352,664). Claims 1-42 have been replaced with new Claims 43-53 to better define the invention.

Carney et al. (US 5,352,664) teach methods using thrombin agonists to promote healing of certain wounds. See column 4, line 65 to column 5, line 6. Chronic ulcers were not among the examples of wounds mentioned in US 5,352,664. Chronic ulcers, as the Examiner points out in citing the HGS Backgrounder, Internet Publication, September 2000 (http://www.hgsi.com/news/press/background_wounds.html; cited as reference U with office action of April 20, 2005) can be, for example, venous ulcers, decubitis ulcers, diabetic ulcers, or arterial ulcers. Unlike the spontaneously healing wounds described in US 5,352,664, chronic wounds do not heal within an expected time frame, and require extensive treatment, which may be medical, surgical, or both.

A chronic skin ulcer presents an entirely different environment biologically from the environment of an excisional wound. For example, the levels of growth factors are reduced in chronic skin ulcers compared to levels found in other wounds. Protease activity is high and prolonged compared to protease activity found in other wounds that heal. See Mast and Schultz, *Wound Repair and Regeneration 4*:411-420, 1996 (copy enclosed as Exhibit A).

Patients who develop chronic skin ulcers are not young, healthy, normally active, and properly nourished. Rather, patients with chronic skin ulcers have underlying conditions that affect the vascular system and healing, for example, diabetes or other chronic disease, immobility, advanced age, or a combination of these factors. See, for example, page 8, column 2, paragraph 3, in the enclosed copy of Chapter 2 in *Chronic Wound Care: A Clinical Source Book for Healthcare Professionals*, Third Edition (Krasner, D.L. et al., eds.) HMP Communications, Wayne, Pennsylvania, 2001 (copy enclosed as Exhibit B).

Healing of a chronic ulcer, if it occurs at all, occurs on a very different time scale from healing of ordinary wounds. See the results of the study presented on page 9, line 21 to page 14, line 16 of the specification. Note that to be included in the study, the patient had to have had the ulcer for ***between 8 weeks and 2 years without healing***. Results are shown in the table on page 12. Note that the majority of the chronic ulcers treated only with saline did not close completely in the treatment period of ***20 weeks***.

The incidence of chronic skin ulcers is expected to increase with an aging and increasingly obese population, while options for treatment of chronic ulcers remain limited. "Despite the number of conservative therapies available, chronic wounds remain a very frustrating problem for health care practitioners. In marketing research conducted by HGS [Human Genome Sciences, Inc., Rockville, Maryland] in late 1998, practitioners expressed considerable frustration with their lack of success in treating chronic wounds, with the time-consuming nature of the regimens they must employ, and with the problems of patient non-compliance they encounter." See website of Human Genome Sciences, Inc., at http://www.hgsi.com/news/press/background_wounds.html dated September, 2000 (cited as reference U with office action).

As such, it is evident that chronic wounds are particularly difficult to heal compared with ordinary wounds. Yet, the subject application provides data showing that one of the recited peptides, TP508, significantly increases the percentage of diabetic wounds which heal compared with untreated controls, and also significantly enhances the rate of healing. The Examiner is respectfully referred to the Table on page 12, which shows that the diabetic wounds of less than half of control patients healed, whereas almost 60% healed in those treated with 10 μg of TP508. The Examiner is also referred to the discussion on pages 13-14 beginning under the heading "Secondary Endpoints," which shows a dramatic decrease in median healing time for those patients treated with TP508 compared with the untreated controls. It is also noted that of the three treatment groups, the most relevant for evaluating the data is the "Per-Protocol" Group (see page 11, lines 21-23).

Because chronic skin ulcers present an environment that is biologically very different from that found in a healing wound, and because chronic skin ulcers occur in populations and

under circumstances that compound difficulty in healing, the effectiveness of agonists of the non-proteolytically activated thrombin receptor in accelerating healing of chronic skin ulcers was far greater than could have been predicted from the teachings of US 5,352,664.

CONCLUSION

The Examiner is requested to consider the above amendments and remarks, and withdraw the rejections. If the Examiner feels that a telephone conference would expedite prosecution of this case, he is invited to call the undersigned attorney.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

By _____

Steven G. Davis
Registration No. 39,652
Telephone: (978) 341-0036
Facsimile: (978) 341-0136

Concord, MA 01742-9133

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Interactions of cytokines, growth factors, and proteases in acute and chronic wounds

BRUCE A. MAST, MD[a,b]; GREGORY S. SCHULTZ, PhD[b]

A healing wound represents a complex series of interactions between cells, soluble mediators, and extracellular matrix. Within this multifaceted environment, there are multiple regulatory points which control the ordered series of events that lead to normal tissue repair. An alteration in this physiologic network can lead to the development of a chronic wound. This article presents an update on the numerous mediators that exist within the wound environment in both acute normal healing and chronic nonhealing wounds. We also present a hypothesis which may provide a conceptual pathophysiologic mechanism with which to understand all chronic wounds. **(WOUND REP REG 1996;4:411-20)**

Numerous invertebrate and amphibian species have the capacity to restore damaged or lost tissue with an exact or nearly exact replica of the original tissue in a process termed regeneration.[1] Mammalian species, however, have largely lost the ability to repair tissue by regeneration except in limited situations in organs such as the liver or in tissues such as the epithelium.[2] In the specific case of a skin wound in mammals, healing occurs by the rapid replacement of damaged or lost tissue with a collagenous scar that acts as a fibrotic spot-weld rather than by regeneration of tissue, a process which typically proceeds more slowly. Thus, healing of skin wounds in mammals may have evolved to minimize life-threatening complications such as infection by rapidly replacing skin tissue with a fibrous scar rather than maximizing appearance and function. In some skin wounds, however, collagenous replacement of tissue is exemplified by pathologic healing resulting in either exuberant fibrotic distortions of appearance or function (keloids, scar contractures) or insufficient replacement of tissue leading to a chronic nonhealing wound.[3]

bFGF	Basic fibroblast growth factor
EGF	Epidermal growth factor
HB-EGF	Heparin-binding epidermal growth factor
IGF-I	Insulin-like growth factor-I
IL-1	Interleukin-1
MMP	Matrix metalloproteinase
PDGF	Platelet-derived growth factor
TGF-β	Transforming growth factor-β
TIMP	Tissue inhibitor of metalloproteinase
TNF-α	Tumor necrosis factor-α

Normal skin wound healing has been the subject of intense research for decades. Data from histologic and cell biology studies have clearly established that numerous types of cells and extracellular components act in an integrated manner to re-establish the integrity of injured tissue. Over the last decade, information on the molecular regulators of wound cells and their actions has substantially expanded our understanding of normal skin wound healing. The model that is emerging emphasizes the incredibly complex interaction of cytokines, growth factors, extracellular matrix components, receptors, and proteases that results in a healed skin wound. This article will review the current knowledge

From the Division of Plastic and Reconstructive Surgery, Department of Surgery,[a] and Institute for Wound Research, Department of Obstetrics and Gynecology,[b] University of Florida, Gainesville, Fla.

Reprint requests: Bruce A. Mast, MD, Division of Plastic and Reconstructive Surgery, JHMHC Box 100286, Gainesville, FL 32610.

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of these interactions in normal skin healing. In addition, we will also present our current understanding of the molecular environment of chronic skin wounds. Lastly, on the basis of cellular, biochemical, and molecular analyses of various chronic wounds, we will propose a new hypothesis that may provide a unifying pathophysiologic mechanism common to all chronic wounds.

CUTANEOUS WOUND HEALING

Wound healing in the skin is a complex biological process in which numerous types of cells, cytokines, growth factors, proteases, and extracellular matrix components act in concert to restore the integrity of injured tissue.[4,5] Cellular, biochemical, and molecular events in tissue repair occur in a time continuum, although for descriptive purposes skin wound healing can be divided into three general phases: (1) the inflammatory phase, (2) the repair phase, and (3) the remodeling phase. There is considerable temporal overlap of these stages of healing, and the entire process lasts for several months.

The process of skin wound healing begins at the moment of tissue injury, and it is the same whether injury is intentional, as in a surgical incision, or unintentional, as in trauma. Damage to blood vessels initiates a hemostatic cascade of blood clotting and platelet aggregation and degranulation. The clot and thrombus form to seal the wound and protect against further bacterial contamination and fluid loss. More importantly, platelet degranulation provides the first regulatory step in repair.[6] Contained within the alpha granules of platelets are several growth factors including platelet-derived growth factor (PDGF), insulin-like growth factor-I (IGF-I), epidermal growth factor (EGF), and transforming growth factor-β (TGF-β). This depot of growth factors released from platelets quickly diffuses from the wound into the surrounding tissue and blood system. TGF-β released from platelets and tumor necrosis factor-α (TNF-α) produced by injured vascular endothelial cells, keratinocytes, and fibroblasts chemotactically draw inflammatory cells into the injured area. This initiates the inflammatory phase which peaks during the first 2 to 3 days.[7]

The acute inflammatory phase is characterized by neutrophils, whereas macrophages predominate after 24 hours. Neutrophils in the wound begin to secrete more pro-inflammatory cytokines (TNF-α and interleukins), engulf and destroy bacteria, and release proteases (elastase and collagenase) that remove damaged and denatured extracellular matrix components. This early burst of protease activity in the wound is important in debriding the wound of damaged matrix and cellular proteins. It is distinct from the later release of matrix metalloproteinases (MMPs) from fibroblasts during the remodeling phase of healing. Circulating monocytes are chemotactically drawn into the wound by TGF-β or fragments of fibronectin and become activated macrophages. Macrophages also secrete pro-inflammatory cytokines including TNF-α and interleukin-1 (IL-1) and engulf and destroy bacteria. The macrophages synthesize and secrete additional growth factors including TGF-β, TGF-α, leukocyte-derived growth factor (a PDGF-like protein), basic fibroblast growth factor (bFGF), and heparin-binding epidermal growth factor (HB-EGF). Under normal circumstances, neutrophils disappear from the wound by about 3 days, probably by undergoing apoptosis, and the inflammatory phase begins to decline. The growth factors secreted locally in the wound by macrophages continue to stimulate migration of fibroblasts, epithelial cells, and vascular endothelial cells into the wound, setting up the next phase of wound repair. A provisional extracellular matrix is created in the tract of the skin wound during the inflammatory phase, which consists predominately of fibrin, fibronectin, and glyocosaminoglycans such as hyaluronic acid. Importantly, uninjured fibroblasts in the dermis adjacent to the wound are stimulated by cytokines and growth factors to begin expressing integrin receptors that specifically recognize fibrin. These fibroblasts can then recognize and migrate into the provisional matrix of the fibrin clot.

As the fibroblasts and vascular endothelial cells migrate into the provisional matrix of the injury site, they begin to proliferate and the cellularity of the wound increases. The repair phase often lasts several weeks. As the number of macrophages in the wound begins to decrease, other cells in the wound such as fibroblasts, endothelial cells, and keratinocytes begin to synthesize and secrete growth factors. Fibroblasts secrete IGF-I, bFGF, TGF-β, PDGF, and keratinocyte growth factor. Endothelial cells produce vascular endothelin-derived growth factor, bFGF, and PDGF. Keratinocytes synthesize TGF-β, TGF-α, and keratinocyte-derived autocrine factor. These growth factors continue to stimulate proliferation, synthesis of extracellular matrix proteins, and angiogenesis. During the repair phase, the early provisional matrix is replaced by a more permanent matrix consisting primarily of collagen, although a noncollagenous "ground substance" is retained composed of glycosaminoglycans and proteoglycans such as chondroitin sulfate and dermatan sulfate.

After the initial scar forms, proliferation and neovascularization cease and the wound enters the remodeling phase which can last for many months. During this last phase, a balance is reached between the synthesis of new components of the scar matrix and their degradation by metalloproteinases such as collagenase, gelatinase, and stromelysin. Fibroblasts are primarily responsible for the synthesis of extracellular matrix components including collagen, elastin, and proteoglycans. In addition, they are also an important source of the MMPs that degrade the matrix. Fibroblasts also secrete the tissue inhibitors of metalloproteinases (TIMPs) which act to block

tissue destruction by MMPs, and they secrete lysyl oxidase which cross links components of the extracellular matrix. In later stages of healing, angiogenesis ceases and capillary density in the wound site decreases. Eventually the scar tissue reaches equilibrium although the mature scar is never as strong as uninjured skin.

It is clear that for a wound to progress from a gelatinous thrombus to a healed wound with a fibrous scar, a complex interplay must occur between pro-inflammatory cytokines, polypeptide growth factors, proteases, inhibitors, and extracellular matrix components. This interplay affects the ultimate outcome of the healing process by regulating and mediating the cellular activity in the wounds and ultimately connective tissue deposition and wound closure.

GENERAL EFFECTS OF TNF-α AND INTERLEUKINS ON WOUND HEALING IN ANIMAL MODELS

The effects of TNF-α on wound healing in animal models are complex and depend on the cell type, the concentration of the cytokines, and the method of analysis. To help determine the importance of various cytokines on initiating the inflammatory state in wounds, endogenous levels of several cytokines were measured in subcutaneously implanted polyurethane sponges in rats.[8] Bioassay measurements of TNF-α and IL-6 showed that levels of both cytokines peaked at day 3 and steadily decreased on days 5, 8, and 13. In contrast, levels of IL-1β were low on days 3 and 5, peaked on day 8, and declined on day 13. IL-2, IL-3, and IL-4 were not detected in the wound fluids. The inverse appearance of TNF-α and IL-1β, combined with the fact that IL-1β synthesis is induced by TNF-α in human fibroblasts, provides strong evidence that TNF-α initiates the pro-inflammatory cascade and induces IL-1β synthesis during skin wound healing.[9]

The actual effect of TNF-α on wound healing was investigated by studying the effects of exogenously added TNF-α in wounds. Steenfos et al.[10] reported that daily injections of relatively low levels of TNF-α (0.6 to 20 ng) into wound chambers implanted under the skin of rats tended to reduce hydroxyproline levels approximately 50%. Additionally, TNF-α injections blocked the increase in hydroxyproline induced by TGF-β by 40%. Mooney et al.[11] found that a single local application of 100 ng of TNF-α into mouse skin incisions increased wound strength approximately 40% compared with controls, but a higher concentration (500 ng) was less effective, having increased wound strength only 20%. In a different experiment, Salomon et al.[12] evaluated the effect of TNF-α on mice with impaired wound healing caused by adriamycin treatment. A single incisional application of 50 ng of TNF-α caused an increase in tensile strength, but application of higher doses (100 ng and 250 ng) caused reduced incisional strength. The detrimental effect of high doses of TNF-α was further established when adriamycin-treated rats were treated with a single local application of 50,000 ng of TNF-α. This large dose of TNF-α significantly decreased wound strength, measured at 7 days after injury in both doxorubicin-treated rats and saline solution–treated rats by approximately 35%. This was also coincident with a decrease in the level of α1 (I) procollagen mRNA in the incisions.

Using the opposite approach of increasing TNF-α levels in wounds, Regan et al.[13] attempted to reduce wound TNF-α levels by application of a neutralizing anti-TNF-α antibody to wounds. Polyvinyl alcohol sponges containing neutralizing anti-TNF-α antibody were implanted under the dorsal skin of mice, resulting in a 77% increase in wound collagen deposition. Low doses of TNF-α applied to the sponge also increased collagen deposition, but the effect was eliminated by treatment with the nonspecific anti-inflammatory drug indomethacin. This indicates that TNF-α indirectly enhanced collagen deposition by promoting a nonspecific low-grade inflammatory response which could be blocked by indomethacin. It is probable that growth factors such as PDGF, TGF-β, and TGF-α synthesized by macrophages drawn into the wound were responsible for the increase in collagen synthesis found in this experimental model.

The role of TNF-α in skin wound healing was also investigated with the use of a strain of mice (C3H/HeJ) that are unresponsive to endotoxin, a potent inflammatory stimulus.[14] Levels of TNF-α protein in wound fluids collected from silicone reservoirs implanted under the dorsal skin of endotoxin-unresponsive C3H/HeJ mice were approximately threefold lower than in wound fluid collected from normal C3H/HeN mice on the first day after surgery. Similarly, the level of collagenase activity in polyvinyl alcohol sponges implanted under the dorsal skin of C3H/HeJ mice was significantly less on day 1 than in sponges implanted in normal C3H/HeN mice. The incision strength in the C3H/HeJ mice was also higher on days 5 and 7 than in normal endotoxin-sensitive C3H/HeN mice, and the levels of procollagen mRNA and protein (hydroxyproline) were also significantly higher in the C3H/HeN mice during the early post-injury period. In summary, these data suggest that low levels of TNF-α produced in normal, noninfected skin wounds may improve healing, but longer exposure to higher levels of TNF-α will reduce tensile strength. This may be due to decreased synthesis of procollagen mRNA and protein, as well as induction of MMPs.

Direct Effects of TNF-α and IL-1β on Various Cellular and Matix Components of the Wound

The direct effects of TNF-α on inflammatory cells, fibroblasts, epithelial cells, and vascular endothelial cells are also important in understanding its overall impact on skin wound healing. First, TNF-α upregulates its own

synthesis by macrophages.[15] Also, TNF-α induces synthesis of IL-1 which can act in synergy with TNF-α to regulate synthesis of collagen, MMPs, and TIMPs.[9] The autoinduction of TNF-α tends to prolong and enhance the effects of low levels of TNF-α produced during the early phase of injury. TNF-α and IL-1β are also both mitogenic for fibroblasts.[16,17] This led to speculation that low levels of TNF-α or IL-1β may contribute to the fibrosis seen in some diseases with unresolved, low-level inflammation such as pulmonary fibrosis, scleroderma, rheumatoid arthritis, and hepatic cirrhosis.[18]

Connective tissue deposition in wound healing can also be influenced by TNF-α because this cytokine affects the synthesis of collagen, MMPs, and TIMPs. Experiments in vitro showed that TNF-α (20 ng/ml, 1.3 nmol/L) and IL-1β (2.5 ng/ml) at low concentrations both decreased α1 (I) procollagen production by 50% in cultured human and mouse skin fibroblasts.[19] The inhibition was due to decreased levels of mRNA for α1 (I) procollagen and was dependent on both the dose and the time of exposure. Tubulin gene transcription was not effected, showing that the effects of TNF-α and IL-1β were gene specific.

Additional experiments with many other types of cells in culture consistently showed that TNF-α and IL-1β directly regulate synthesis of MMPs and TIMPs in vitro. In cultures of normal human synovial fibroblasts, TNF-α and IL-1β both induced expression of MMP-9 and MMP-1.[20] TNF-α enhanced collagenolysis in cultures of human uterine cervical fibroblasts by increasing production of collagenase (MMP-1) and stromelysin (MMP-3) and decreasing synthesis of TIMP.[21] In this cell culture system, IL-1β increased synthesis of MMPs and TIMP coordinately. Similar results were reported for cultures of human endometrial stromal cells where both TNF-α and IL-1β stimulated secretion of MMP-1, MMP-3, and MMP-9, but not MMP-2, in a concentration-dependent manner.[22] TNF-α treatment also increased production of MMP-1 and MMP-3 in a dose-dependent manner, but suppressed production of TIMP-1 in cultures of human chorionic cells.[23] In addition, TNF-α induced MMP-9 in cultures of bovine pulmonary microvascular endothelial cells which caused increased microvascular permeability by degradation of ECM.[24] In summary, TNF-α and IL-1β directly stimulate synthesis of MMPs in numerous types of cells including skin fibroblasts while simultaneously decreasing synthesis of TIMPs. Furthermore, these influences on MMPs and TIMPs have been shown to have secondary effects on connective tissue metabolism.

Effects of IL-1β on Skin Wound Healing

The cytokine IL-1β has many effects on cells that are similar to TNF-α. In particular, IL-1β and TNF-α both increase mitosis of fibroblasts.[17,25] In addition, IL-1β was reported to increase proliferation of smooth muscle cells and vascular endothelial cells and to be a chemoattractant for neutrophils and macrophages. As described here previously, both IL-1β and TNF-α increase MMP production by fibroblasts and macrophages. These biological effects of IL-1β prompted evaluation of IL-1β in animal models of wound healing. When applied to infected acute wounds in rats, IL-1β led to a significantly increased rate of wound healing compared with vehicle-treated controls.[26] IL-1β was also evaluated in a phase II, randomized, placebo-controlled, double-blind study of healthy volunteers with surgically created split-thickness wounds.[27] Wounds treated with 0.5 mg IL-1β/day achieved complete healing faster than wounds treated with placebo. Higher and lower doses showed no difference in healing times. Conversely, a phase II study of pressure sores showed no beneficial healing effects of IL-1β.[28] Doses of IL-1β of 0.01 mg, 0.1 mg, and 1 mg per square centimeter of ulcer area did not show acceleration of healing.[28]

In summary, results from these in vitro and in vivo experiments show that TNF-α and IL-1β have important effects on healing of skin wounds. The effects of TNF-α are dependent on concentration and duration of exposure. Given once at low levels, TNF-α can moderately enhance healing of skin wounds by indirectly stimulating inflammation and increasing growth factors produced by macrophages. However, TNF-α given at high levels or for extended periods of time has a detrimental effect on healing of skin injuries. The detrimental effects appear to be due to a combination of TNF-α actions. First, TNF-α upregulates its own synthesis and induces synthesis of IL-1β, which tends to amplify and prolong the effects of low doses of TNF-α. Second, both TNF-α and IL-1β suppress synthesis of extracellular matrix proteins and TIMPs while increasing synthesis of MMPs. In the environment of a wound, these effects of TNF-α could lead to high levels of TNF-α, IL-1β, and MMPs.

COMPARISON OF ACUTE AND CHRONIC WOUND ENVIRONMENTS

We have measured the levels of several pro-inflammatory cytokines and their antagonists in a series of fluids collected from acute and chronic human wounds.[29] The pro-inflammatory cytokines TNF-α and IL-1β were present in high levels early during normal skin wound healing, but there was a fairly regular and consistent reduction in their levels as healing proceeds. A more precise measure of the significance of these levels is provided by analysis of the cytokines and their antagonists. One such antagonist is p55, the soluble form of the TNF-α receptor protein which retains the ability to bind TNF-α and prevents TNF-α from activating its cellular receptor. Levels of p55 were consistent in acute wound fluid samples collected daily for 8 days after surgery.

When compared with TNF-α levels during the same period, the ratio of p55/TNF-α in acute wound fluids was about 6 to 1 in favor of p55 (300 versus 50 pg/ml). Similarly, the levels of IL-1ra, the natural inhibitor of IL-1α and IL-1β, were also similar in the 21 acute wound fluid samples analyzed. The ratio of IL-1ra to IL-1β in acute wound fluids was about 320 to 1 in favor of the IL-1ra (8000 versus 25 pg/ml). In summary, these data indicate that the ratios of antagonist to inflammatory cytokines for TNF-α and IL-1β in molecular environment of acute wounds strongly favor the inhibitors which suggests that the biological effects of TNF-α and IL-1β are closely regulated in acute healing wounds.

In chronic wound fluids, the pattern of these cytokines was much different from those in acute wounds. The levels of the pro-inflammatory cytokines TNF-α and IL-1β were more variable, but the average levels of TNF-α (500 pg/ml) and IL-1β (2500 pg/ml) were about 100-fold higher than in mastectomy fluids. Levels of IL-6 (30 pg/ml) were twofold to fourfold higher in chronic wounds than the levels measured in late acute wound fluids, whereas levels of IL-8 were low and similar in chronic and acute wound fluids.

Levels of p55 in chronic wound fluids were substantially higher than in acute wound fluids (approximately 1700 versus 300 pg/ml). Nevertheless, the ratio of p55/TNF-α in chronic wound fluids had decreased twofold to about 3 to 1 in favor of the inhibitor, p55 (1700 versus 500 pg/ml). In contrast, the average level of IL-1ra in chronic wound fluids was lower than in acute wound fluids (approximately 3000 versus 8000 pg/ml). The decrease in IL-1ra caused the ratio of IL-1ra to IL-1β in chronic wound fluids to decrease from 320 to 1 in acute wounds to about an approximately equal ratio of 1.2 to 1 in chronic wounds. Therefore, it appears that the biological activity of these cytokines should be significantly greater in chronic wounds.

Mitogenic growth factors

Several studies have analyzed fluids collected from acute and chronic wounds for their effects on proliferation of fibroblasts in culture to assess the mitogenic potential of the wound environments. Cell culture studies showed that supplementation of standard culture medium with 5% fluid collected from full-thickness acute skin wounds in pigs significantly increased growth of normal human dermal fibroblasts.[30] The highest mitotic activity was present in wound fluid collected on day 1 after injury and decreased on days 2 and 3. The mitogenic activity was inhibited in a dose-dependent manner with neutralizing antibodies to PDGF. Addition of neutralizing antibodies to bFGF also reduced the mitogenic stimulation of the wound fluid. Fluids collected from partial-thickness excision injuries generated similar results. Thus, fluids collected from acute full-thickness and partial-thickness skin injuries in pigs were mitogenic for fibroblasts because of the actions of PDGF and bFGF. Marikovsky et al.[31] also detected HB-EGF in fluid from partial-thickness pig wounds. This newly described member of the EGF family of growth factors, synthesized by macrophages, was present in highest quantities on day 2, with progressive reduction to day 8.

Several studies have analyzed fluids collected from human wounds. Katz et al.[32] collected fluids from split-thickness skin graft donor sites covered with a vapor-permeable barrier. Culture medium supplemented with a 2% solution of this acute human wound fluid resulted in a significant increase in growth of human dermal fibroblasts and umbilical vein endothelial cells. We also have analyzed acute wound fluids collected from mastectomy incisions and from split-thickness skin graft donor sites.[29] Our results showed that fluids from both types of acute healing wounds stimulated growth of all three major types of skin cells (i.e., fibroblasts, keratinocytes and capillary endothelial cells). Furthermore, measurement of growth factors in these acute fluids indicated that TGF-α, EGF, TGF-β, and IGF-I were present in the fluids at physiologically significant levels. It is well established in the literature that all four of these growth factors are mitogenic for cultured skin fibroblasts and all except TGF-β also are mitogenic for epidermal cells.[33]

The profile of growth factors in chronic wounds is somewhat different. Cooper et al.[34] analyzed chronic wound environments by measuring levels of growth factors and cytokines in extracts of porous dextran beads placed in pressure ulcers overnight. They reported detecting PDGF-AB, bFGF, and EGF in most of the 20 chronic pressure wound fluid samples but could not detect TGF-β in 17 of 20 samples. We found that levels of EGF in chronic wounds were substantially lower than in acute wound fluids, whereas levels of TGF-α and TGF-β were similar in both types of wounds and levels of IGF-I were higher in chronic wounds.[29]

Chronic human wound fluids also have been analyzed for biological effects on wound cells. Alper et al.[35] collected fluids from cutaneous ulcers under vapor-permeable membranes. Addition of the fluids to culture medium at a 2% level caused the fibroblasts to round up and detach from the dish. Similar results were reported by Bucalo et al.[36] who reported that fluids collected from chronic venous stasis ulcers inhibited the serum-stimulated proliferation of human dermal fibroblasts and failed to stimulate the proliferation of vascular endothelial cells. Data from our laboratory also show that fluids from human chronic wounds are not mitogenic and block DNA synthesis stimulated by serum and acute wound fluids.[29]

Proteases and protease inhibitors

Agren et al.[21] used pigs to determine the profile of MMP-1 in three distinct types of surgical wounds. They re-

ported that extracts of normal skin contained low levels of MMP-1, whereas surgical incisions showed a peak MMP-1 content on day 1 and thereafter steadily declined. Granulation tissue from large unsutured full-thickness wounds showed a high content of MMP-1 on day 5 then a sharp decline to day 7 followed by a slow decline to day 21. Partial-thickness wounds had peak levels of MMP-1 activity on days 3 to 5 which approached the low levels of normal skin by day 7 when epithelialization was complete and the scab was sloughed. This important study showed that MMP-1 activity peaks during active healing and declines to extremely low levels as the wound heals.

Wysocki and Grinnell[37] analyzed fluids from acute and chronic human wounds for proteases and evaluated the effects of the wound fluids on fibronectin, a protein that is important for cell adhesion and wound repair. They found that fibronectin was intact in fluids from acute injuries (i.e., suction blisters and mastectomy incisions). In contrast, fibronectin in fluids from two venous stasis ulcers was partially degraded and was totally degraded in fluids from two diabetic ulcers. Intact fibronectin added to the chronic wound fluids in vitro was quickly fragmented, indicating the presence of proteases in the fluids. Furthermore, wound fluids which contained extensively degraded vitronectin and fibronectin reversibly inhibited cell adhesion. They attributed the destruction of fibronectin to neutrophil elastase and proposed that protease activity in chronic wound fluids causes degradation of adhesion proteins and prevents cell adhesion necessary for normal wound closure.

Wysocki et al.[38] extended these initial investigations of proteases in acute and chronic wound fluids. Estimating MMP levels from densitometric scans of gelatin zymograms, they reported that fluids from four patients with chronic wounds had fivefold to tenfold increases in levels of MMP-2 and MMP-9 compared with levels in mastectomy fluids. Stromelysin-1 mRNA and protein, which is induced by IL-1β, also was identified in keratinocytes during wound healing and in fibroblasts in the base of chronic wounds.[39]

The elevated levels of MMP activity could be due to a combination of increased levels of MMPs and decreased levels of TIMPs. Howard et al.[40] analyzed fluids from nine patients with mastectomies and two patients with chronic pressure ulcers for TIMP-1 using a radioimmunoassay. They reported that serum levels for TIMP-1 were 147 ± 5 ng/ml. Levels in mastectomy fluids were significantly higher and peaked on day 2 after surgery at 1528 ng/ml then decreased on day 10 to 734 ng/ml. In contrast, TIMP levels of fluids from the two chronic wounds were lower at 377 ng/ml. These findings are consistent with results from our laboratory.[29] Analysis of 20 mastectomy fluids for protease activity with the use of Azocoll as the substrate indicated that the average level of protease activity was 0.75 μg/ml with a range of 0.1 to 1.3 μg/ml. In contrast, the average level of protease activity in 32 chronic wound fluids was 87 μg/ml with a range of 1 to 584 μg/ml. Addition of ethylenediamine tetraacetic acid or the synthetic MMP inhibitor, Ilomostat, significantly reduced the levels of protease activity in the chronic wound samples to about 15% of the total protease level, indicating the presence of MMPs. Levels of TIMP were inversely related to the levels of MMP activity. We also have analyzed samples of acute and chronic human wound fluids for protease activity using different substrates. These include gelatin zymography, casein zymography, Azocasein proteolysis assay, a neutrophil elastase assay, a neutrophil elastase inhibitor assay, and a cathepsin G assay. The results of these assays consistently show that, compared with acute wound fluids, chronic wounds contain markedly elevated levels of different types of proteases and reduced levels of protease inhibitors.

Growth factor and growth factor receptor degradation

In light of the observations of elevated protease activity and diminished mitogenic activity of chronic wound fluid, we also evaluated the stability of growth factors and their receptors in acute and chronic wound fluids.[29] We found that iodinated EGF was stable in acute wound fluid with only 2 of 20 mastectomy fluid samples causing any measurable degradation of the growth factor. In contrast, 14 different chronic wound fluid samples all caused substantial destruction of the added EGF. Furthermore, growth factor degradation was inhibited to levels that were similar to that of acute wounds when chronic wound fluids were treated with ethylenediamine tetraacetic acid or Ilomostat. Similar results were obtained for degradation of PDGF and IGF-I with the use of the same samples of acute and chronic wounds. Additionally, the EGF receptor was found to be stable in mastectomy wound fluid, whereas it was substantially degraded in chronic wound fluid. Again, the degradation was blocked by the addition of the MMP inhibitors, Ilomostat, and ethylenediamine tetraacetic acid.

CHRONIC WOUND PATHOPHYSIOLOGY

Most skin wounds heal without difficulty, but in some people with predisposing factors, an acute wound fails to heal resulting in a chronic wound. There are several types of chronic wounds that are recognized clinically, including diabetic foot ulcers, decubitus ulcers, venous stasis ulcers, and ischemic ulcers caused by arterial insufficiency. Treatment of chronic wounds and the lost productivity of persons with chronic wounds are a significant drain on the work force and health care resources of the United States. Current treatments for chronic wounds are only partially successful and are generally limited to nonspecific and generalized at-

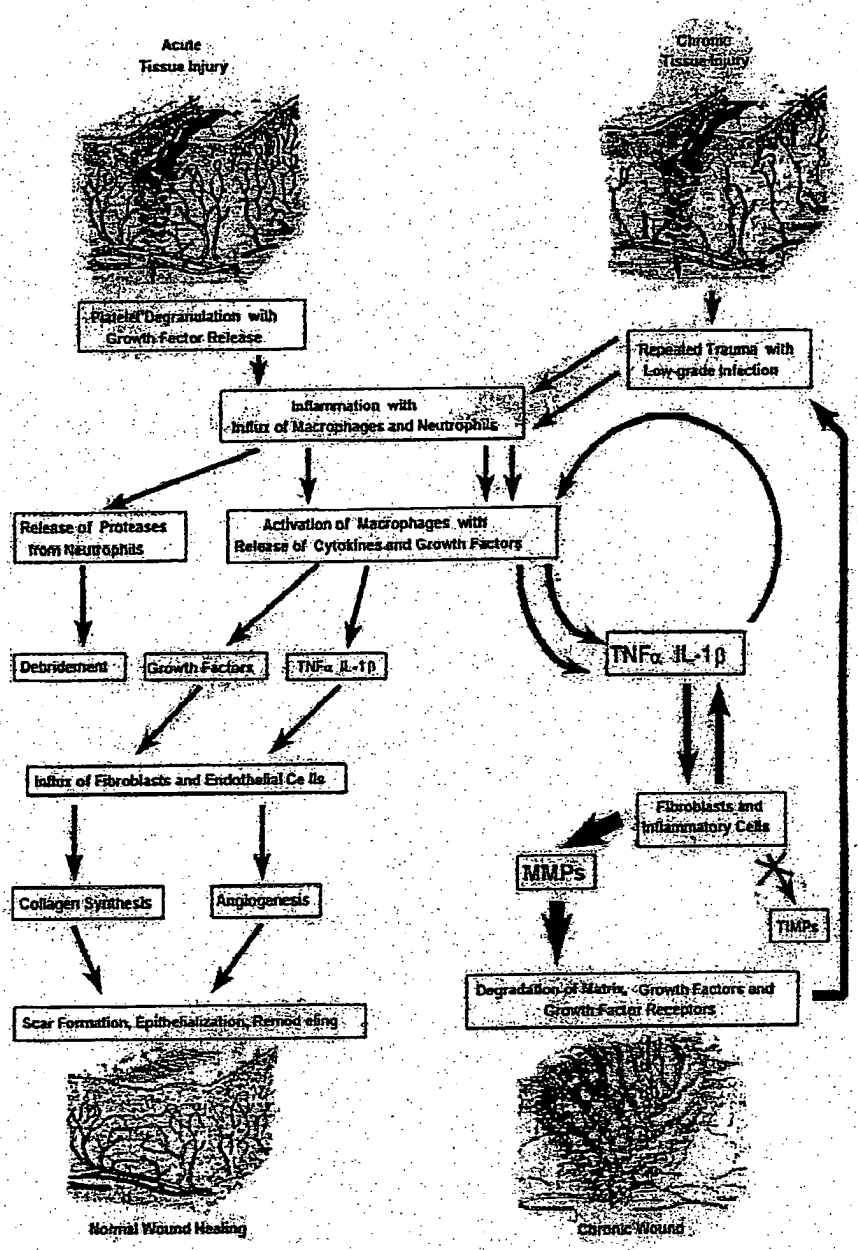

Figure 1 Model for chronic wound pathophysiology.

tempts to reduce the conditions that initially lead to and propagate the injury. For example, the most frequently used treatments include physical devices such as special shoes which are intended to reduce tissue trauma, antibiotics which reduce bacterial contamination, and dressings which are designed to remove necrotic or ischemic tissue. However, even with the appropriate use of these agents, chronic wounds frequently fail to heal or heal slowly probably because these dressings and treatments do not actively alter the pathophysiologic characteristics of most chronic wounds. Ideally, therapy from chronic wounds would be aimed specifically at the molecular pathophysiology responsible for the failure of the wound to heal.

A common origin for the failure of wounds to heal

Several differences in the molecular environments of acute and chronic wounds have been identified that may play roles in the pathophysiology of chronic wounds. Specifically, we and others have found elevated pro-inflammatory cytokines, high protease activity, and diminished growth factor activity in chronic wound compared with acute healing wounds. On the basis of these observations, we have formulated a hypothesis that integrates these profiles into a common molecular pathophysiology of chronic wounds (Figure 1).

Outwardly, chronic wounds appear to be rather heterogenous: diabetic foot ulcers, pressure ulcers, venous stasis ulcers, and ischemic ulcers. However, all these

chronic wounds are similar in that each is characterized by one or more persistent inflammatory stimuli: repeated trauma, ischemia, or low-grade bacterial contamination. Eventually, the skin barrier is broken and bacterial colonization occurs. Inflammatory molecules from bacteria such as endotoxin, platelet products such as TGF-β or fragments of extracellular matrix molecules such as fibronectin stimulate inflammatory cells (neutrophils and macrophages) to enter the wound. These activated inflammatory cells then secrete inflammatory cytokines such as TNF-α and IL-1β which synergistically increase production of MMPs while reducing synthesis of TIMPs. The elevated MMP activity degrades the ECM which interferes with cell migration and connective tissue deposition. MMPs also degrade growth factors and their target cell receptors which further limits the progression of the wound healing cascade by eliminating the mediators of the cascade. Entry into the repair phase is thereby impaired, and the wound fails to heal. This differs from acute wounds in that there is a limited pro-inflammatory stimulus rather than ongoing stimulation as proposed in chronic wounds.

Examining each of the major clinical classes of chronic wounds, similarities in molecular response can be noted. Diabetic foot ulcers typically occur when there is repeated trauma to an area of the foot because of lack of sensation caused by neuropathy. This leads to tissue ischemia and tissue injury with breakdown of the skin which leads to secondary bacterial contamination from the open wound. Tissue fragments and bacterial products such as endotoxin (lipopolysaccharide) stimulate inflammation and release of pro-inflammatory cytokines. Pressure ulcers (decubitus) have a similar pattern of events. Repeated tissue trauma, primarily ischemia, occurs in insensate areas when the pressure in the tissue exceeds capillary perfusion pressure. This leads to the sequence of tissue breakdown, bacterial contamination of the open wound, and inflammation. Venous stasis ulcers are characterized by venous hypertension and edema in the lower extremity. This results from faulty venous valves which produce elevated venous pressure within the leg. When the venous pressure exceeds the capillary perfusion pressure of skin, local ischemia occurs. When this is combined with minor local trauma, the sequence of tissue breakdown, bacterial contamination of the open wound, and secretion of pro-inflammatory cytokines occurs. Ischemic ulcers of the lower extremity secondary to arterial insufficiency follow a similar pattern. Minor trauma leads to an open wound which is slow to heal because of poor oxygen perfusion. Bacterial contamination of the open wound occurs and inflammation follows.

Once these different types of wounds reach the point where there is an open wound with low-level bacterial contamination, we believe they enter a common cascade pathway which prevents healing. The next step is the prolonged secretion of TNF-α and IL-1β by inflammatory cells (macrophages and neutrophils) and other wound cells such as keratinocytes, fibroblasts, and vascular endothelial cells. This prolonged secretion of TNF-α becomes amplified because TNF-α stimulates its own synthesis and the synthesis of IL-1β. The elevated levels of TNF-α and IL-1β directly stimulate production of MMPs by macrophages, fibroblasts, and keratinocytes, whereas TIMP production is inhibited. These proteases degrade essential ECM components, integrin receptors, growth factors, and growth factor receptors, all of which retard healing.

It is important to note that both acute and chronic wounds begin with a similar pro-inflammatory cytokine response to the tissue injury which includes release of TNF-α and IL-1β. In healing wounds, however, tissue injury is a single occurrence, and the stimulus for the inflammatory cytokine is limited and transient. The normal sequence of inflammatory events then follows with neutrophils and macrophages removing bacteria and denatured ECM components from the wound. The macrophages, fibroblasts, vascular endothelial cells, and keratinocytes secrete growth factors which promote epithelialization, ECM production, angiogenesis, and scar formation. However, in wounds which fail to heal, tissue injury is recurrent, and this prolongs and amplifies the pro-inflammatory cytokine cascade leading to elevated protease activity and impaired growth factor actions which impairs healing.

Protease levels in sequential chronic wound fluids

To further evaluate the validity of this hypothesis, we analyzed an initial series of sequential chronic wound fluids in collaboration with Drs. Mike Stacey and Naomi Trengove at Fremantle Hospital in Australia. Wound fluids were collected from 10 patients with venous stasis ulcers before treatment and collected again after 2 weeks of therapy when the ulcers were beginning to heal. If the above hypothesis is correct, one would predict that protease activity would decrease in the wounds as healing occurred. To standardize the collection process, these patients were hospitalized the night before treatment and no fluids were consumed after midnight. At 8:00 a.m. the next morning, patients drank 1 L of water, the ulcer was covered with an occlusive dressing, and the leg was placed in a dependent position. After 1 hour the fluid that had spontaneously collected was removed and was then analyzed for protease activity with the use of Azocoll hydrolysis. The average level of Azocoll hydrolysis was high in the 10 samples collected before therapy began (52 ± 12 mg protease/ml). After 2 weeks of treatment, the levels of Azocoll hydrolysis decreased in 8 of 10 of the patients to an average level of 15 ± 6 mg protease/ml. Overall, this is a 3.4-fold decrease in protease levels of the 10 patients. The MMP inhibitor, Ilomostat, inhibited almost all the Azocoll hydrolysis activity in

the sequential wound fluids. For example, Ilomostat decreased the average Azocoll hydrolysis activity by 99.2% from 52 to 0.4 mg protease/ml in the samples collected before treatment began. Ilomostat also effectively decreased protease activity in fluids collected 2 weeks after treatment began from 15 to 0.4 mg/ml. Therefore, it was concluded that essentially all the protease activity in the sequential wound fluids detected by Azocoll hydrolysis assay were metalloproteinases. All the patients showed clinical signs of granulation tissue formation and epithelialization although none had fully healed in the 2-week period. Thus, it appears that a reduction in elevated protease activity in chronic venous stasis ulcers is correlated with conversion of a nonhealing, chronic wound into a healing wound.

CONCLUSIONS

The complexity of normal skin wound healing is exemplified by the coordination of cellular and extracellular components which provide the body with a healed wound. Inflammatory cytokines, mitogenic growth factors, molecular receptors, extracellular matrix components, cell adhesion molecules, proteases, and enzyme inhibitors all interact in a well-orchestrated manner. The complexity of the system is compounded by the overlap of many of these components, such that the absence of one component is sometimes inconsequential because another growth factor or protease can carry the load. Despite these safe-guards, pathologic healing is encountered with an uncomfortable degree of frequency. Comparison of acute healing wounds and chronic nonhealing wounds provides a greater understanding of the importance of the interaction of the various components in the wound. Furthermore, on the basis of these new insights, it is possible to propose a new hypothesis that provides a molecular mechanism for the pathophysiology that may be common to a variety of chronic wounds. As more work is done, and greater understanding achieved, it may be possible to treat pathologic healing with therapy that is directed at the molecular level.

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Chapter 2

Chronic Wound Healing and Chronic Wound Management

Dean P. Kane, MD, FACS, CWS

Objectives

After reviewing this chapter, the reader should be able to:

1. Identify the basic and complex mechanisms of wound healing.
2. Recognize the environments necessary to maintain homeostasis in the human wound ecosystem.
3. Manage a patient's comorbidities with a curative goal when appropriate.
4. Provide care in a compassionate, palliative manner when cure is not possible.

Case Vignette

Ms. N. Legg was a 59-year-old loving grandmother with a 25-year history of left lower-extremity venous insufficiency. For 15 years, elevation of her leg and compression therapy resolved the aching, varicose veins, and minor ulcerations. About 10 years ago, however, the ulcers enlarged affecting her ability to work; as a result, greater attention to wound management through cleansing, care, and occasional oral antibiotics was needed. Unfortunately, even with vein ligations, the leg developed more ulcers, swelling, and woody lymphedema, and the patient required multiple hospitalizations for cellulitis. Ultimately, a split-thickness skin graft and compression therapy closed her ulcer, which remained closed for two years. Ms. Legg eventually returned to the outpatient wound clinic with a new ulcer. She remained independent, employed, and ambulatory, but her ulcer would not improve despite optimal compression and elevation. The wound care clinicians tried debridements, including topical enzymatic agents and mechanical debridement, platelet-derived growth factor therapy, topical antiseptics and antibiotics, topical oxygen therapy, and hyperbaric oxygen therapy, but nothing would fully control the weeping, ulceration, and dermatitis, nor reduce the four hours of wound care needed daily. Protein, calories, vitamins and minerals, and anti-oxidants were provided to Ms. Legg, and endocrine deficiencies were reversed. Eventually, however, insurance reimbursement was denied; pharmaceuticals were also denied due to medicolegal concerns. Funding Ms. Legg's wound care was difficult, despite the care, support, and wound care supplies offered to Ms. Legg by the wound care clinic. In spite of the wound care clinic team's sincerest desires to salvage her leg, Ms. Legg finally healed with an above-the-knee amputation.

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Introduction

The most common chronic wounds include lower extremity ulcers, diabetic ulcers, and pressure ulcers. Other types of chronic wounds include skin cancers, nonhealing surgical wounds, fistulae, dermatitis or vasculitis wounds, radiation wounds, and burns. Differentiated from acute wounds that heal in a matter of days or weeks, chronic wounds may persist for months or years and occasionally can last a lifetime. Every chronic wound drains the life and resources of the individual and our society.

In the United States, there were an estimated 2.1 million pressure ulcers in 1990 alone with a national expenditure of 1.3 billion dollars.[1] It is estimated that in the next 15 years the population over the age of 65 in the United States will double from 35 million to 60 million senior citizens and quadruple for citizens over the age of 85 from 4 million to 17 million individuals.

With decreasing healthcare funding and a dynamic move of ill or chronically wounded patients from the acute hospital setting to long-term care, an explosion of chronic nonhealing wounds is expected. The development of mobile and flexible chronic wound services will be essential to achieve curative or palliative wound care, as well as an improvement in the understanding of the quality-of-life issues, which are just beginning to be fully appreciated today.

A chronic wound is a window to underlying disease. Each wound is a symptom of underlying infirmities that undermine the potential for healing.

—Dean Kane, MD

A Window to Underlying Disease

Wound care was first documented following ancient Egyptian war injuries in the Edwin Smith Papyrus of 1600 BC. Since then, wounds continue to be treated with powders, salves, gauze, and now hydrogels, hydrocolloids, alginates, and so-called advanced dressings. Such passive topical remedies actually have one common denominator—to provide an appropriate environment for healing wounds.

In prior centuries, antiseptics were used to fight infection. The 20th century saw the use of antibiotics with more specificity for bacterial infections. Approaches of the 21st century will stimulate the host to produce the anabolic repair factors necessary for wound healing.

Passive and interactive dressings have ushered in the bioactive dressings of the 21st century. Not dissimilar to autogenous skin grafts or autogenous composite tissue flaps, these biologic dressings provide cover and yield growth factors, which stimulate wounds to heal. These bioactive dressings represent a paradigm shift in our understanding of wound healing and wound management; rather than simply provide an appropriate healing environment, the wound minienvironment is actually stimulated to heal.

The wound environment is part of a larger human life ecosystem. A chronic and complex wound represents a symptom or an uncompensated response to a much larger problem given the comorbidities of the individual's physiologic state. No wound stands alone. It is important to appreciate the macroenvironment, which sustains stable wound repair. Such healing potential[2] is also dependent upon reversing or managing comorbidities. A chronic wound is a window to underlying disease. Each wound is a symptom of underlying infirmities that undermine the potential for healing. These comorbidities include direct and indirect factors. Direct factors affecting healing potential include arterial insufficiency, chronic illness, diabetes, radiation injury, skin cancer, surgery, trauma, and venous insufficiency. Indirect factors affecting healing potential include dementia, immobility, malnutrition, neuropathy/ insensibility, radiation therapy, and a suboptimal wound environment.

Advanced Wound Caring

Wound healing is a complex process influenced by the host (the patient), the environment, and the healthcare professional. Despite the tenacity of many chronic wounds and the resultant frustration felt by patients, caregivers, and healthcare professionals alike, with perseverance, many of these wounds can be healed. For patients where healing is not anticipated, compassion-

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ate, palliative wound management can prevent deterioration of the wound and provide comfort to the patient. The goals of advanced wound caring are to improve the outcomes and quality of life of those individuals suffering with chronic and complex wounds while reducing costs and providing comfort.

Human Wound Physiology: Overlapping Environments

Traditional wound management consisted of stuffing wounds (and their unique minienvironments) with pastes and fabrics. Other environments exist, such as nanoenvironments (DNA, RNA, and protein metabolism), microenvironments (intercellular activity), and macroenvironments (the human life system, lifestyle, and surroundings), which all overlap to maintain life and well being.

Each niche of the human body has developed a unique, balanced microenvironment in order to perform optimally. Each microenvironment is encased within a larger ecosystem known as a macroenvironment or the body as a whole. This form and function are unique, whether a mitochondrion, a cell, an organ, or the whole human life system. Microenvironments are upset by disease or wounds that expose them to outside environments or by changes in internal ambiance, which can reset the parameters of cell function. Homeostasis is the balance of life as a whole. In order for a wound or ulcer to heal, the entire life system must return to physiological balance. Each disease resets the internal balance, which at some set point or in addition to other infirmities will unbalance the playing field of healing.

Cellular activity is the basic activity that makes life possible. Each cell survives by the mitosis of itself and symbiosis with other cells. Each is its own microenvironment, sustaining itself by the action of other, equally interdependent cells. In the wound environment, the platelet stimulates the neutrophil, which calls for the macrophage. The macrophage orchestrates the harmonious interactions of function-specific cells, such as the angiocyte, neurocyte, fibroblast, myoblast, melanocyte, keratinocyte, and countless others.

Nanoenvironmental factors affect the building and breakdown (anabolism and catabolism) of the host. This nanoenvironment includes our genetic make up. Ultimately, gene expression provides the chemical messengers (proteins, hormones, cytokines, growth factors, and others) that deliver the appropriate stimulus or messages to guide and harmonize wound repair, organ regeneration, and restoration of function and well being. Form and function of each cell integrate into a human life ecosystem. Our ability to reach for sustenance, create shelter, and develop creative thought is the anabolic (build and repair) mechanism of human survival.

TABLE 1: COMPLICATIONS RESULTING FROM CHRONIC WOUNDS

- Infection
- Increased length of healing, length of hospital stay, and cost of healing
- Pain
- Disability and loss of work productivity
- Catabolism
- Deformity
- Disuse atrophy and loss of function
- Reduced self esteem
- Loss of limb
- Loss of life

In our modern age of technologic marvels, we are the longest living humans with the lowest death rate. Yet we have made little progress in delaying the threshold of disease and its consequences. This leaves humans with a longer disease span and, therefore, more years of infirmity and greater economic burden. Following sexual maturation, our progeny take over where we have left off. It is the human being's unique *joie de vivre* to search for eternal youth and with it continuous anabolism rather than evolutionary catabolism.

Aging—the Common Thread of Disease

For the improvements of age have but little influence on the essential laws of man's existence – Thoreau

Traditional medical paradigms are changing. Disease management treats illness as separate and fragmented entities. Any one disease is only one part of a whole human life system. There must be a common thread. This common thread appears to be the process of aging.

Aging does not appear to be an active process expressed by a particular gene or genes. Aging appears

TABLE 2: THE GOALS OF CHRONIC WOUND MANAGEMENT

1. Saving life
2. Saving limb
3. Infection control
4. Pain management
5. Complete and durable wound healing of a stable chronic wound
6. Optimal function
7. The best cosmetic result
8. Cost efficiency

to be a passive process of declining hormone expression and entropy following sexual maturity. Thereafter, each one of our physiologic systems fails. Since each of our physiologic systems is interdependent, they each affect each other. The weakest link causes a disease to which we succumb. If no system is a weak link, we age gracefully and die of old age.[3]

Today, the average American woman lives to 79 years of age, and her male counterpart lives 74 years. During the next 50 years, the median human lifespan is expected to double as it did during the last 100 years. Fifty percent of "baby boomers" are expected to celebrate their 100th birthday. Public sanitation, vaccines, and antibiotics have led to this longer lifespan. The biologic discoveries and new technologies of the last 50 years are expected to push maximum lifespans beyond their historic 120 years. Human biologic therapy in the form of balanced nutritional supplements and antioxidants, balanced hormone replacement therapies, and, in the near future, gene therapy will not only increase maximum lifespan, but also decrease the disease span, that interval of time where infirmity and age-related diseases diminish our quality of life.[4]

Studies using human growth hormone (and other therapies) have shown dramatic results, including increased endurance, immunity, and many other enhancements to quality of life.[5]

The Anti-Aging Medicine Model

Current concepts in anti-aging medicine dovetail with an holistic approach to preventive chronic wound management. As a functional and physiologic approach to medicine, an anti-aging approach optimizes the well being of the individual to delay age-related diseases and their consequences, such as chronic wounds. It is felt that 90 percent of chronic degenerative diseases, such as atherosclerotic arteriovascular disease, adult-onset diabetes, aging-related cancers, and dementia, which causes neurologic immobility, incontinence, and other manifestations, are caused by aging. With the average threshold of disease beginning at age 50 and median lifespan at 75, a widening gap of infirmity and debility is expected as baby boomers age. Without effort to reverse or forestall those diseases associated with aging, such as lifestyle changes, balanced hormone replacement therapies, and gene therapy, the personal and public burdens of aging upon our society are expected to explode.[6]

The human genome project with the sequencing of over 100,000 genes that express each constituent of the individual is nearly complete. Nutrient-rich and calorie-restricted diets are known to prolong the lifespan of animals.[7] Technology promises great breakthroughs but not without the personal involvement of each person and family member.

Oxidative stress caused by living longer, physiologic and environmental stressors, and deficiencies found in nutrient-poor refined foods of the day lead to a longer span of disability and greater degenerative diseases of aging.[7] Supplemental vitamins and anti-oxidants are used to re-balance this oxidative-stress. There are those who recommend anti-oxidant supplementation beginning in childhood where its long-term impact would be greatest.

The Hayflick phenomenon, which observed a defined number of cell divisions prior to cell senescence and death, has identified the potential for telomere lengthening correlated to prolonged life but not necessarily greater quality of life.[8]

Prevention and Treatment

Tell me and I forget. Teach me and I remember. Involve me and I learn. —Benjamin Franklin

Prevention is based on anticipatory diagnosis. If one can predict the risk of a chronic wound problem before it occurs based on family history, past medical history, and mental, physical, and laboratory examination, then we can quantify risk and take appropriate steps for prevention. Similarly, once a wound has occurred, based on these assessments we can create a management plan

to cure or palliate the patient's wound.

Primary prevention is based on maintaining aging at a level of repair above the threshold of disease, that point where irreversible physiologic change occurs. Complicated wounds are just one of many irreversible changes created by disease. Chronic and complex wounds are, therefore, a symptom of one or many system failures such as diabetic neuropathy, arterial insufficiency, venous stasis, malnutrition, immobility, and other direct and indirect physiologic factors.

Secondary prevention is the cognitive action of identifying a disease and reducing the risk of its progression once it already has been manifested.

As we unfold the mysteries of aging, we will find higher thresholds of anabolic and physiologic function, stalling the degenerative diseases of aging that cause the personal, economic, and social burdens of chronic wounds.

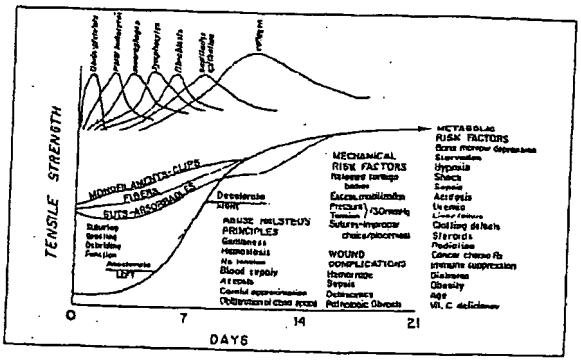


Figure 1. *The wound healing curve: The sequence of healing in spontaneous soft tissue repair. Note the acceleration (left shift) of the wound healing process and deceleration (right shift), which will modify the chronic wound environment.*[9]

Acute Wounds

When the skin is injured, its normal barrier function is breached. A complex cascade of inflammation termed the "wound healing curve" initiates protection of the host internal environment and its organs. In the healthy immunocompetent host, acute traumatic abrasions, lacerations, and superficial skin and soft tissue injuries heal spontaneously without complications through the four normal phases of the wound healing curve: hemostasis, inflammation, proliferation, and remodeling (Figure 1).

Chronic Wounds

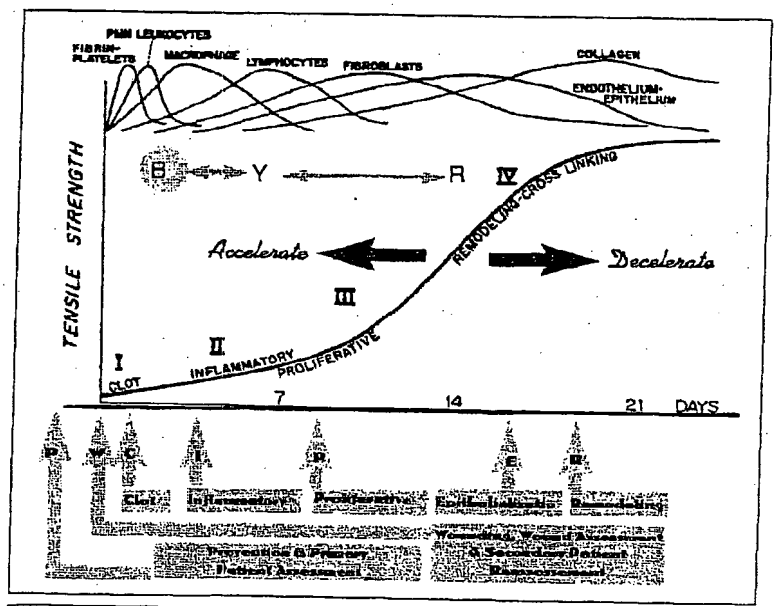
If the natural healing progression is delayed, a chronic wound results. In 1992, Lazarus, et al., defined chronic wounds as those that "fail to progress through a normal, orderly, and timely sequence of repair or wounds that pass through the repair process without restoring anatomic and functional results."[10]

Today, considering wound healing as the only goal of management is short sighted. Each wound and each host are individual with unique problems and potential for healing and productivity. The goals of chronic wound management include wound healing with stability and return to optimal function with the least pain and least healthcare expenditure (Table 2).

A more holistic view of wound healing is necessary to meet the patient's need for tissue perfusion, nutritional support, mobility, incontinence management, pain control, rehabilitative conditioning, and maintenance of self esteem in order to achieve improved outcomes of greater function and aesthetic appearance. Adopting these holistic goals of form and function will allow the wound care specialist to best address the unique requirements of each patient. Careful consideration of wound care versus wound repair and consideration of the unique social, economic, and medical factors of each patient is warranted if we are to return patients to their greatest functional potential.

Reconstructive options should be considered for those selected patients whose length of healing would be extraordinarily long (in months or years) or whose health status is appropriate for more rapid healing options.

Today, many patients with chronic debilitating neurologic maladies, malnutrition, and immobility may never experience a healed chronic wound. While ongoing assessment and wound maintenance are necessary, these patients may never become suitable candidates for reconstruction. For these patients, compassionate local management and holistic comfort care with dignity are reasonable goals. Many times, patient comfort and quality of life supersede aggressive intervention despite the attendant risks of limb loss, pain, and death. These are issues that must be considered with each patient, family, and primary care physician.



Prevention & Primary Patient Assessment
Anti-aging medicine
Vitamins, Minerals and Anti-oxidants
Nutrition
Protein, Caloric Restrictions
Balanced Hormone Replacement Therapies
Thyroid, anabolic (growth, testosterone) etc
Gene Therapy
Pressure Relief / Off loading
Bed / Chair surfaces
Mobility
Incontinence correctio n
High risk evaluation: perfusion, mentation, etc.

Wound Assessment & Secondary Patient Reassessment
Management of Differential Dx:
Pressure sore - pressure relief
Arterial wound - revascularize
Venous insufficiency - elevation, compression
Diabetic ulcer - glucose tolerance, AB, unloading
Fungal ulcer - drier enviroment, anti-fungal Rx
Skin CA - Bx, excision, RTX
Trauma and Surgery -
appose edges / tensionless closure'
nutrition, rehabilitation for ADL's, proper suture selection
Burn - hydrate, NSAID, cool, debride, graft, therapy
Reversal of co-morbidities:
dehydration, malnutrition, immobility, radiation, incontinence, etc (see Table 5)

Clot Phase
evacuation of hematoma
control: coumadin, NSAID, Vitamin E

Inflammatory Phase
antibiotic po / iv / topical
antiseptics: bacitracin, bactroban, betadine, chlorpactin, gantamycin, neomycin, silvadene, etc
debridement
elevation
vitamin A
pressure relief / off-loading
growth factors
HBO
Warm-Up Therapy (R)

Proliferative Phase
normal saline
hydrocolloid
skin graft
tissue flap
nutrition
hydrogel (amorphous)
growth factors
biologic skin equivalents

Epithelialization Phase
hydrogel (water)
film dressings
skin graft
biologic skin equivalents

Remodeling Phase
silicone gel sheeting
pressure
steroid injection
compression
elevation
radiation therapy
collagenase / proteases

Figure 2. *Matching wound therapy to the wound healing phase. Combining the wound healing curve (Figure 1) with chronic wound management by each phase of healing will allow the advanced wound health manager greater aptitude in prevention and reversal of chronic wounds.*

Infection

The germ is nothing; it is the terrain in which it is found that is everything
—Louis Pasteur

Wound infection is divided int three categories: colonization, loc invasion or dermatitis, and distar travel or sepsis.

Bacteria colonize all wounds; mos of these are not virulent and cause n invasion, multiplication, nor a inflammatory response. Virulent an invasive bacteria, such as *Pseudomona aeruginosa*, *Staphylococcus aureus*, an *Streptococcus*, may cause invasion infection, morbidity, limb loss, and mortality at concentrations less than 10^5 colonies/gram of tissue. Control o infection or sepsis is critical.

When invasion of an offending organism occurs, the cardinal signs of localized infection develop. The cardinal signs of infection include rubor (erythema), calor (warmth), tumor (induration), and dolor (pain).

The host's armies of antibodies and immune cells have been partially overwhelmed. Many times, local antiseptics or antibiotics will level the immunologic playing field back toward healing. Other times, debridement or more invasive surgical debulking of the bioburden is necessary. In this case, reconstruction or resurfacing is postponed until healing balance is obtained, as risk of graft and flap failure is high.

It is impossible for any of the commonly used antiseptics to have an appreciable effect in sterilizing a wound...the greatest benefit is to be obtained by aiding the physiological agencies, which bring about the natural recovery from infection.
—A. Fleming

Sepsis or total host invasion car-

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TABLE 3. THE WOUND BUILDING TABLE: A COMPARATIVE ANALOGY OF WOUND HEALING CELLS AND HOUSE BUILDING CONTRACTORS

Phase	Goal	Principle Wound Cell	House Building Contractor
I	Hemostasis	Platelets	Capping off offending conduits
II	Inflammation	Neutrophils	Unskilled laborers to clear the site
III	Proliferation	Macrophages	Supervisor cell
	Granulation	Lymphocytes	Specific preparers of the site
		Angiocytes	Plumbers
		Neurocytes	Electrician
	Contracture	Fibroblasts	Framers
		Keratinocytes	Roofers and siders
IV	Remodeling	Fibrocytes	Remodelers

ries with it a high risk of morbidity and 50 percent of graft or flap failure. The patient's total immune defenses have been compromised, and he or she may succumb to this hypermetabolic response and multiorgan system failure. Multidrug therapy is often necessary, including antibiotics, nutritional assistance, occasional steroids, and life-saving therapies when shock occurs. If the bioburden at the wound site is great, gram stain and culture and sensitivity, drainage, debridement, and the occasional amputation may be lifesaving.

Wound Healing from a Builder's Perspective

Healing a wound is much like building a house (Plate 1, Page 745). Various steps must be organized and synchronized. Without the proper signals at the correct time, neither a house would be built nor a wound healed. If the correct laborers (cells) and the appropriate work materials (nutritional substrate) are not present in the correct amount, the house (wound) will be weak and incomplete.

Wounding destroys the various layers of the skin and the underlying soft tissues just as a hurricane or bombing destroys a house and its landsite (Table 3). Arteries, veins, and nerves become exposed just as plumbing and electric conduits are exposed. The initial phases of construction are to cap these conduits in order to prevent further destruction and loss. This is the clot or hemostatic phase of wound healing. Nonskilled workers, similar to nonspecific neutrophils, then clean up the landfill during the inflammatory phase.

The proliferative phase is dependent upon the macrophage, just as building a home is dependent on a supervisor. Without each, building or wound healing stops or is delayed. Once the supervisor or macrophage has established itself at the site, it signals the other subcontractors or specific immune and repair cells to begin their expert work. Lymphocytes are more specific preparers of the wound site. Fibroblasts begin the framing or reinforcement of the wound. Angiocytes and neurocytes lay the conduits for perfusion and communication just as plumbers and electricians perform their jobs. At last, the epithelial cells (keratinocytes), like roofers, pro-

If you do not control the infection, the infection will control the host.
—Dean Kane, MD

TABLE 4. MODIFICATIONS IN CHRONIC WOUND HEALING

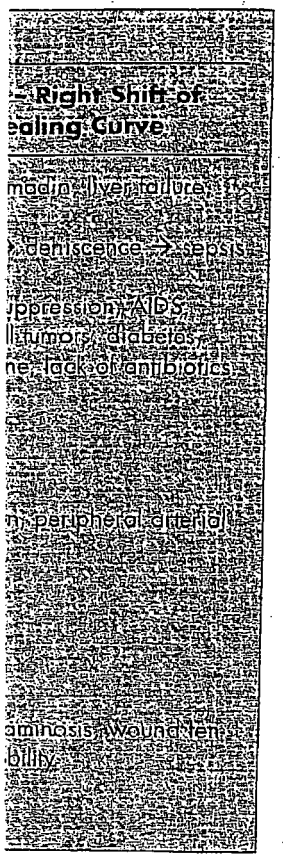
Phase	Acceleration – Left Shift of Wound Healing Curve	Deceleration – Right Shift of Wound Healing Curve
I Hemostatic	Cautery, pressure, vitamin K	ASA, NSAIDS, Coumadin, liver failure, thrombocytopenia, DIC → hematoma → dehiscence → sepsis
II Inflammatory	Debridement, antibiotics, ↑perfusion (↑ Hgb, ↑cardiac output, drugs), ↓RBC sludging, Retin-A, ↓O2 free radicals, gentle tissue handling, venous decongestion, limb elevation, leeches, drugs, growth factors	Transplant immunosuppression, AIDS, leukopenia, stem cell tumors, diabetes, steroids, poor hygiene, lack of antibiotics
III Proliferative	Close dead space, linear wound healing, approximate edges, skin grafts, flaps, vitamins, nutrition, proper sutures, staple, Round wound healing, moist sterile occlusive environment, protection from incontinence	Diabetes, malnutrition, peripheral arterial disease, edema
IV Maturation	Wound immobility for hypertrophic scars, pressure, excision, silicone gel sheeting, nitrates, anabolic steroid, radiation therapy, combination of above	Malnutrition, hypovitaminosis, wound infection, unprotected mobility

vide the outside barrier to the home or wound. Over the next two years, remodeling of the wound occurs in order to reduce scar quantity while increasing scar strength and quality.

Communication or chemotaxins signal the proper cascade of wound healing events. At any time, any phase may be interrupted. When this occurs, the chronic wound develops. Certain conditions, such as diabetes, autoimmune diseases, and immunoincompetence due to medications, such as steroids or anti-rejection drugs, will delay the full inflammatory response of wound healing.

Without the building materials of adequate nutrition, including protein, carbohydrate, fat, fluid, vitamins, and minerals, wound-healing delays occur. Granulation tissue, the foundation of the wound, is dependent on angiogenesis and neurogenesis. Without the foundation, the house cannot be built nor the wound healed. Without hormones, growth factors, cytokines, and other cell messengers to stimulate granulation tissue formation, the wound will not heal. Continued anabolism of the host must be maintained in order to achieve healing during the fibroblast and fibrocyte stages of collagen formation when scaffolding for the repair of the wound site is required. Fibroblasts are subcontractors in the building process, providing the scaffolding for myoblasts to pull together and strengthen the wound. As these last cellular subcontractors leave the wound site, continued energy and a clean work environment are necessary for the keratinocytes to finish the external barriers.

Large amounts of energy, resources, time, and money are necessary in chronic wound healing. Reconstructive surgery may be a cost-effective and efficient option for selected patients. If a roof is lost or damaged during a hurricane or tornado, it will be replaced or repaired. Similarly, if the epithelium is injured, as in a deep burn, a deep sloughing injury, or a venous ulcer, a skin graft is used to repair the lost epithelial surface. When deeper structures are lost, such as skin and subcutaneous tissue during wide excision of skin cancers, superficial ulcers, or traumatic injury, then approximating the wound edges or carrying adjacent tissues from redundant skin areas



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while still attached to their random blood supply can hasten healing by primary intention. Perfusion and the cells needed for wound healing are more readily available so healing is more rapidly achieved then when healing occurs by secondary intention. This is much like building a split trailer house and riveting the edges with collagen.

While each of these diagnoses will be further expanded upon within this source book, it should be realized that each wound or ulcer is created by unique physiologic events. Accurate assessment and diagnosis of each wound and its underlying factors are essential in order to achieve optimal healing.

AHCPR Guidelines

The Agency for Health Care Policy and Research (AHCPR) has developed guidelines on the prevention and care of pressure ulcers.[11] These guidelines were organized into six diagnostic and management recommendations based on the available science at the time they were written. While specific to the management of pressure ulcers, these are excellent guidelines for the prevention, care, and cure of all chronic wounds.

Prescriptives

While the U.S. Food and Drug Administration (FDA) does not regulate most dressings and therapeutic modalities, each intervention carries with it risks, benefits, indications, and contraindications not dissimilar to any drug prescribed. It is incumbent upon the wound care practitioner to fully explore and provide the least expensive but most effective dressing, offloading surface, and/or therapeutic management style available to each individual. Many expensive antibiotics are no more effective than over-the-counter (OTC) antibiotics. Many patients are unable to afford expensive yet less time-consuming primary or secondary dressings, and the advanced wound practitioner must remain flexible, creative, and eager to help manage these problematic decisions while facilitating patient care. Each of these therapeutic decisions will modify the overlapping environments of each wound. Routine assessment as determined by the gravity and magnitude of the wound are essential as we follow the ulcer through its wound-healing curve.

TABLE 5. THE DIFFERENTIAL DIAGNOSIS OF CHRONIC WOUNDS

- Trauma
- Burns
- Bites and stings
- Venous ulcers
- Diabetic ulcers
- Pressure ulcers
- Arterial ulcers
- Connective tissue disorders
- Malignancy

The Timing of Wound Management: A Common Pathway in Wound Healing

You can observe an awful lot by just watching – Yogi Berra

When to do what is a considerable source of confusion for most wound care clinicians. A firm knowledge of the timing of wound management (Figure 2) and modifications in chronic wound healing (Table 4) is essential to understand advanced wound management. The concept of matching the dressing to the wound is based upon understanding the different wound-healing phases a chronic wound undergoes. A more complete appreciation of the wound healing curve will allow the clinician a greater aptitude in preventing and reversing wound development and rescue of surgical graft and flap wound healing failures.

Hastening wound healing (acceleration or left shift of the wound-healing curve) is predicated on prevention or reversal of complications, such as hematoma, infection, malnutrition, and other factors. Delayed wound healing (deceleration or right shift in the wound-healing curve) will increase length of hospital stay, increase chronicity of the wound, diminish self esteem, increase pain, and increase cost. In Table 4, the columns labeled acceleration and deceleration in chronic wound healing describe those factors during the healing phase that will enhance or diminish wound healing. These factors may be medical or surgical in nature, but they provide insight to the great array of management options available for assisting patients in developing durable, painless wound coverage.

Split-thickness skin grafts are used for stage 2 and granulated stage 3 wounds of nonpressure origin where wound cover without durability is needed. Well-vascu-

larized flaps of skin, fat, fascia, muscle, and occasionally bone are used to reconstruct the larger defects created over bony prominences. Anticipatory diagnosis and risk assessment are necessary when any skin graft, flap resurfacing, or reconstruction is planned. Improved prognosis for healing would incorporate preoperative planning for optimization of flaps and grafts as further expanded in the chapter of surgical repair.

As we better understand the bigger picture in wound management, we realize the unique evolving and dynamic nature of aging, disease, anabolism, and catabolism of each of our patients. Based on the wound-healing curve and anticipatory diagnosis of high-risk patients for wounding and healing potential, we can match the needs of the patient with his or her phase in wound healing. Figure 2 shows which management is appropriate for each phase of wound healing.

Anticipatory prevention by optimizing the functional capacity or functional reserve of our patients will delay the onset of irreversible disease, such as stroke, heart attack, diabetes, or hip fracture. These patients require diagnosis of potential health risks and education on the lifestyle or medical changes necessary to reverse that risk. In the anti-aging scheme of things, laboratory panels, histories, and physical exams may identify patients at risk for cardiovascular disease, diabetes, cancer, osteoporosis, and many other morbid illnesses that lead to chronic wounds. Modifying lifestyle changes, such as the appropriate supervised use of antioxidants, nutrition, fitness, and hormone-related therapies, has maintained the activities of daily living and quality of life necessary to attain functional capacity above the threshold of disease in many patients. Once wounding has occurred, both the wound and the patient must be assessed in order to optimize the overlapping environments and the direct and indirect factors causing the wound.

The Paradigms: They Are A-Changing

The times they are a-changing. – Bob Dylan

The paradigms in chronic wound management are changing. There has been an obvious shift in surgical, medical, and therapeutic options in chronic wound care. Older and more acutely ill patients present challenges as our society ages. Reimbursement disincentives are cost shifting more chronically patients to lesser skilled facilities, including t home. Less surgery is being offered as patients a considered less optimal for reconstructive optior On the other hand, there is a greater awareness a knowledge of factors positively impacting healir potential. Marked technological strides using stim latory growth factors and biologic skin substitut have reduced cost and improved healing when use appropriately.

Conclusion

The cheapest wound is no wound. The next cheapest woun is a healed wound. – Source unknown

Let us not forget the most basic tenets of chron wound management as we treat our patients cor passionately. Every wound has multiple underlyir factors that created it. Chronic wounds are sym toms of underlying disease. The challenge presente by each wound and each patient is unique. Optim wound care involves individualizing standar approaches based on evolving recommendatior and the social and financial challenges of eac patient. While the process is challenging, it is qui rewarding. As our society ages, better understandir of the degenerative diseases associated with agir and their manifestations, such as wounds, wi become more popular and important. In the futur primary prevention and cellular manipulation age-related diseases will reduce the enormous grov ing burden of chronic wounds to a minimum. In th meantime, more comprehensive management an compassionate care for wound patients is essential.

Case Vignette Wrap Up

In the case of Ms. Legg, the clinicians at the woun care clinic found that wound healing was compose of overlapping environments: nano-environmer (DNA and protein), micro-environment (cellular mini-environment (wound), and macro-environmer (host and environment). The direct and indirect fa tors affecting viability and healing potential of he chronic wound were explored. Every attempt to com pensate for the catabolic needs of each of these esc lating environments was met with resistance and ult mate frustration. After 10 years of a chronic and com

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plex nonhealing wound, the above-the-knee amputation was the most compassionate and functional choice for Ms. Legg. She returned to her husband, her family, and a stable job following rehabilitation with an improved and pain-free quality of life.

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Questions

1. The overlapping phases of durable wound healing include:
A. Arterial ulcers, venous insufficiency, diabetic wounds, pressure ulcers
B. Hemostasis, inflammation, proliferation, remodeling
C. Prevention, assessment, planning, management
D. Rubor, calor, tumor, dolor

2. The goals of chronic wound healing include all except:
A. A durable, healed wound
B. Compassionate care and stable, pain-free, non-infected wound
C. Higher insurance reimbursement
D. Greater self-esteem and return to a productive lifestyle

3. Infections include all of the following except:
A. Osteomyelitis
B. Cellulitis
C. Sepsis
D. Immunosuppression

Answers: 1.B; 2.C; 3.D

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Co-Edited by:

Diane L. Krasner, PhD, RN, CWOCN, CWS, FAAN
George T. Rodeheaver, PhD
R. Gary Sibbald, BSc, MD, FRCPC (Med/Derm), MACP, DABD

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Accurate indications, adverse reactions, and dosage schedules for wound care products and drugs are provided in this book, but it is possible that they may change. The reader is urged to review the package information data of the manufacturers of any products mentioned.

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